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FATTY ACID COMPOSITION AND THERMAL BEHAVIOR OF NATURAL SPHINGOMYELINS

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Summary

We found significant differences in the fatty acid composition of several bovine brain, egg yolk and sheep erythrocyte sphingomyelins. These differences in fatty acid composition influence the thermal behavior of hydrated sphingomyelin as recorded by differential scanning calorimetry. Significant differences were also found in the temperature and complexity of the order-disorder phase transitions of bovine brain sphingomyelin obtained from different sources which, in general, correlate with the relative content of the saturated fatty acids (palmitic ($C_{16:0}$) and stearic acid ($C_{18:0}$) acids) and the long unsaturated nervonic acid ($C_{24:1}$).

Introduction

The fatty acid composition (chain length, degree and position(s) of unsaturation, isomeric position, etc.) of phosphatidylcholines from different tissues has been well characterized [1]. There is much less known about natural sphingomyelins, although they also exhibit a mixed fatty acid composition. The situation is less complex than for glycerolipids since there is only one fatty acid/sphingomyelin molecule. However, sphingomyelins may also vary, albeit much less extensively, in their sphingosine backbone [2]. The major sphingosine consists of an 18-carbon chain with a *trans* double bond at the 4 position. At least sixty different species of sphingosine bases have been described [2], many of which are found in sphingomyelins from bovine milk and bovine kidney [2]. Bovine brain and many other sphingomyelins consist predominantly of the $C_{18:1}$ sphingosine base and small amounts of $C_{18:0}$ dihydrosphingosine and/or $C_{20:1}$ sphingosine [3].

We have examined the thermal behavior of bovine brain sphingomyelin and demonstrated a broad, complex order-disorder transition at a relatively high temperature (30—40°C) [4]. In the present paper, the fatty acid compositions of bovine brain sphingomyelin from different suppliers, as well as egg yolk and sheep erythrocyte sphingomeylins, have been determined. In addition, we have demonstrated how the differences in fatty acid composition sphingomyelin influence their thermotropic behavior as determined by scanning calorimetry.

Materials and Methods

Bovine brain sphingomyelin was purchased from the following: Lipid Products (Surrey, U.K.), United States Biochemical Co. (Cleveland, OH), Avanti Biochemical (Birmingham, AL) and Research Products International (Elk Grove Village, IL). Egg yolk and sheep erythrocyte sphingomyelin were purchased from Avanti Biochemical and Supelco (Bellefonte, PA), respectively. Thin-layer chromatography (TLC) of these lipids developed in $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v/v) and of *n*-butanol/$CH_3CH_2OH/CH_3COOH/H_2O$ (8 : 2 : 1 : 3, v/v/v/v) showed them to be more than 99% pure.

Bovine brain sphingomyelin was also isolated from bovine brain, purchased from a local slaughterhouse (Acme Boneless Beef Co., Wilkinsonville, MA), by a modified version of the method described by Folch et al. [5]. The crude lipid extract was flash evaporated to dryness and redissolved in 140 ml 0.1 N NaOH in CH_3OH. This procedure hydrolyzes most of the *O*-acyl ester phospholipids. After 1 h at 37°C, 860 ml $CHCl_3$ and 10 ml 0.04% $CaCl_2$, plus an upper phase consisting of $CHCl_3/CH_3OH$/0.04% $CaCl_2$ (3 : 48 : 47, v/v/v) were added, the mixture shaken vigorously and allowed to separate. The lower phase was washed again with new upper phase, then flash evaporated to dryness. The lipid residue was resuspended in chloroform and placed over a silicic acid column (Bio Sil A 100—200 mesh) and monitored by TLC (silica gel/F-254 plates, E. Merck, Darmstadt, F.R.G.) developed in $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v/v), detected by iodine and by ninhydrin spray (Merck).

Samples of sphingomyelin were weighed in tubes with a central construction. An excess amount of doubly distilled water (40—50% of the total weight; phospholipid plus water) was added to each dry mixture using a microsyringe. The tubes were purged with nitrogen and immediately flame sealed. Equilibration was accomplished by repeatedly centrifuging the mixture through the central constriction at 50°C. Immediately after opening the tube, a well-mixed sample was placed in a differential scanning calorimetry pan. Samples containing approximately 8 mg (sphingomyelin + water) were scanned at a heating rate of 5°/min in a Perkin-Elmer (Norwalk, CT) DSC-2 scanning calorimeter. Transition enthalpies were calculated by measuring the peak area with a planimeter and correlating with the enthalpies of known standards, such as gallium and dimyristoyl phosphatidylcholine.

Fatty acid methyl esters were prepared by methanolysis with anhydrous HCl according to the method of Kishimoto and Hoshi [6]. The dried sample was placed in a tightly closed tube with methanolic HCl for 18 h at 75°C. The methyl esters were extracted with hexane for gas-liquid chromatographic analysis. Gas-liquid chromatography was performed on a Hewlett-Packard

(Avondale, PA) 5710A gas chromatograph with a 5711 flame ionization detector. Separation was achieved using a 6 ft, 2 mm inner diameter glass column packed with 5% diethyl glycol succinate (DEGS PS) on chromosorb WHP 100/120, programmed from 165 to 200°C at a rate of 2 degrees/min.

Results and Discussion

The fatty acid compositions of the different sphingomyelin samples are shown in Table I. As expected, significant differences in fatty acid composition are observed between sphingomyelins isolated from bovine brain, egg yolk and sheep erythrocytes. Egg yolk sphingomyelin is extremely rich in palmitic acid (approx. 86%), whereas sheep erythrocyte sphingomyelin is abundant in nervonic acid ($C_{24:1}$), approx. 55%. However, the extreme differences observed for the batches of sphingomyelin all isolated from bovine brain was unexpected. At least three divisions may be made on the basis of fatty acid composition. The two samples a and b (Table I) are similar and are relatively rich in the two saturated fatty acids palmitic and stearic (approx. 60—70% combined) with lesser amounts of nervonic acid (approx. 12%). Sample d (Table I) is again rich in stearic acid but contains very little palmitic acid; in this case nervonic acid becomes a major constituent, accounting for approximately 30% of the fatty acids. The two samples f and g (Table I) are similar in that their major fatty acid is nervonic acid (65—75%). They differ in that in one case palmitic acid is the most abundant saturated fatty acid and no stearic acid is present; in the other sample stearic acid becomes a major fatty acid constituent at the expense mainly of palmitic acid.

This degree of variability in fatty acid composition of isolated sphingomyelins led us to examine the thermal behavior of fully hydrated samples. Differential scanning calorimetry heating curves of the seven different samples are shown in Fig. 1. Clearly both the shape of the differential scanning calo-

TABLE I

FATTY ACID COMPOSITION AND TRANSITION ENTHALPHY OF SPHINGOMYELINS

(a) Bovine brain (Research Products International); (b) bovine brain (United States Biochemical); (c) egg yolk (Avanti Biochemical); (d) bovine brain sphingomyelin prepared by authors; (e) sheep erythrocyte (Supelco, Inc.); (f) bovine brain (Avanti Biochemical); (g) bovine brain (Lipid Products).

Fatty acid	Source						
	a	b	c	d	e	f	g
16 : 0	18.1	16.0	86.2	2.0	9.5	19.8	1.9
18 : 0	50.1	42.5	6.0	40.5	1.5		24.1
22 : 0	7.4	5.8	3.0	1.2	18.7	3.3	3.9
23 : 0	2.0	2.2			5.1		
24 : 0	8.2	9.5		10.4	10.9	3.5	5.1
24 : 1	12.9	11.5	4.9	29.9	55.1	73.4	64.7
22 : 6	1.2	13.2		10.3			
26 : 0				5.7			
ΔH (kcal/mol)	8.5	9.1	5.9	6.2	5.3	5.5	5.6

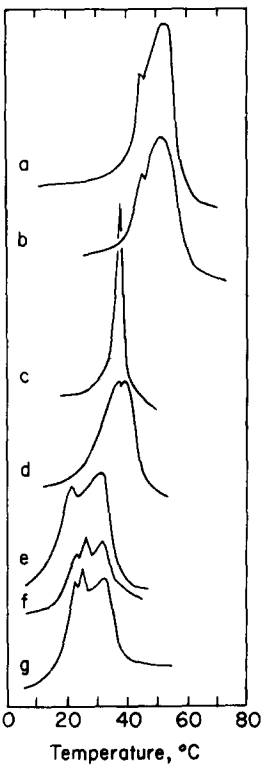

Fig. 1. Differential scanning calorimetry heating curves of hydrated sphingomyelins (approx. 50 wt.% H_2O) scanned at 5°C/min. (a) Bovine brain (Research Products International); (b) bovine brain (United States Biochemical); (c) egg yolk (Avanti Biochemical); (d) bovine brain prepared by authors; (e) sheep erythrocyte (Supelco, Inc.); (f) bovine brain (Avanti Biochemical); (g) bovine brain (Lipid Products).

rimetry curves and the transition endotherm maxima vary significantly. Bovine brain sphingomyelin samples a and b show virtually identical differential scanning calorimetry curves with a major transition peak maximum at approx. 52°C and a discrete shoulder (peak maximum approx. 42°C). These two samples are very similar in fatty acid composition, with almost one-half of the total fatty acid content being stearic acid ($C_{18:0}$). This probably explains why these two sphingomyelin preparations undergo unusually high order-disorder transitions. The major transition at approx. 52°C agrees well with that reported by Barenholz et al. [7] for synthetic stearoyl sphingomyelin (T_m = 52.8°C). The bovine brain sphingomyelin prepared by the authors (sample d) also has $C_{18:0}$ as its major fatty acid but it also has an appreciable amount of 24 : 1 (approx. 40% 18 : 0 and approx. 30% 24 : 1). This sphingomyelin shows a broad order-disorder transition with peak maxima at 36°C and 39°C (Fig. 1d), in good agreement with values reported previously [4,7]. Thus, an increase in $C_{24:1}$ content mainly at the expense of $C_{16:0}$ results in both a lowering of the transition temperature and the presence of two peak maxima approximately equivalent in heat capacity. The transition temperatures of nervonoyl ($C_{24:1}$) sphingomyelin is approx. 25—30°C (Calhoun, W.I. and Shipley, G.G., unpublished results). A phase rich in this low melting $C_{24:1}$ sphingomyelin

presumably segregates and may be responsible for the lower melting transition (peak maximum 36°C). The higher melting peak is presumably due to a stearic acid-rich sphingomyelin phase that contains significant amounts of the $C_{24:0}$ and $C_{24:1}$ sphingomyelins. One might expect these two sphingomyelin species to lower the transition temperature of the stearic acid-rich phase since their transitions occur at lower temperatures (see above and Ref. 7). The lower melting behavior of these sphingomyelins is probably due to the fact that for chain lengths of 22 : 0, 24 : 0 and 26 : 0, the chain lengths of the fatty acid and sphingosine moieties become significantly different. Thus, the fatty acid chain could extend 6—10 carbons further into the lipid bilayer, causing a perturbation to the bilayer sufficient to lower progressively the order-disorder transition.

Bovine brain sphingomyelin samples f and g contain unusually high amounts of nervonoyl sphingomyelin. The differential scanning calorimetry behavior of the two samples are very similar (Fig. 1f and g) and also quite complex. Three discrete endotherm maxima are observed at approximately 22, 26, and 32°C and the differential scanning calorimetry curves are very similar to those shown by Barenholz et al. [7]. Again, this complex melting behavior is probably due to demixing of sphingomyelin phases differing in fatty acid composition and melting behavior.

Egg yolk sphingomyelin undergoes a single sharp order-disorder transition at approx. 39°C (Fig. 1c). Palmitoyl ($C_{16:0}$) sphingomyelin is the predominant species (approx. 86 wt.%). This correlates well with previous calorimetric studies of hydrated $C_{16:0}$ sphingomyelin bilayers which show a single sharp transition at approx. 41°C [7,8].

Sheep erythrocyte sphingomyelin undergoes a broad thermal transition with peak maxima at 21°C and 31°C (Fig. 1e) which is quite similar to that of the bovine brain sphingomyelin samples f and g. This sphingomyelin has a very low content of stearic and palmitic acids, but is unusually rich in behenic ($C_{22:0}$) acid and nervonic acid (greater than 50 wt.%). Again, the presence of nervonic acid as the predominant fatty acid results in a rather low order-disorder transition temperature. However, the influence of behenic acid as the major saturated fatty acid on the melting behavior remains to be determined. It should be noted that sphingomyelin is the dominant phospholipid present in sheep erythrocyte membranes [9] and its physical properties may have a significant effect on the overall characteristics of that membrane.

Thus, there appears to be a general pattern which relates the fatty acid composition of sphingomyelin to its thermal behavior. The palmitic acid and stearic acid-rich sphingomyelins undergo an order-disorder transition at a relatively high temperature (Fig. 1a and b). In comparison, the sphingomyelin preparations which contain approx. 65—73 wt.% nervonic acid exhibit a much lower, albeit more complex, thermal transition (Fig. 1f and g). The sphingomyelin prepared by the authors (sample d) has a broader fatty acid profile, and undergoes its order-disorder transition at intermediate temperatures. The simple sharp melting behavior of egg yolk sphingomyelin reflects its relatively simple fatty acid composition dominated by palmitic acid.

We have demonstrated: (1) the importance of the fatty acid composition in determining both the complexity and the overall thermal behavior of various

hydrated sphingomyelin bilayers *, and (2) that the fatty acid profile of bovine brain sphingomyelins obtained from different suppliers can vary significantly. Whether the latter observation is due to the selection of fractions collected during purification or represents a real variation in the fatty acid composition of bovine brain sphingomyelin remains to be determined.

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* At present we have not taken into account possible effects due to the variation in the sphingosine base. These effects would probably be secondary to those due to the large variations in fatty acid composition since bovine brain sphingomyelin consists of 85—90% $C_{18:1}$ sphingosine, the remaining 10—15% being $C_{18:0}$ sphingosine and $C_{20:1}$ sphingosine [3,7]. Egg yolk sphingomyelin is approx. 97% $C_{18:1}$ sphingosine [3].